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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/563,774	07/13/2006	Mehmet Toner	022727-0138	3248

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EXAMINER	
KOSSON, ROSANNE	
ART UNIT	PAPER NUMBER
1652	

NOTIFICATION DATE	DELIVERY MODE
05/16/2008	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

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Office Action Summary	Application No.	Applicant(s)	
	10/563,774	TONER ET AL.	
	Examiner	Art Unit	
	Rosanne Kosson	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *03 April 2008*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,8-17,19-23,26-30,32-35,37-41,43-45 and 58-64* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,8-17,19-23,26-30,32-35,37-41,43-45 and 58-64* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

The amendment filed on April 3, 2008 has been received and entered. Claims 1, 8-17, 19 and 28 have been amended. Claims 2-7, 18, 24, 25, 31, 36, 42 and 46-57 have been canceled. Claims 58-64 have been added. Accordingly, claims 1, 8-17, 19-23, 26-30, 32-35, 37-41, 43-45 and 58-64 are examined on the merits herewith to the extent that they read on the elected invention.

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Claim Rejections - 35 USC § 112, first paragraph

Claims 1, 8-15, 19-23, 26-28, 32-35, 37-39 and 43-45 are again rejected, and claims 58-60 and 62-64 are rejected, under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. Specifically, the claims recite a method of preserving a cell (amended from a biomaterial having a membrane) and a transporter molecule or transporter protein in which the cell is exposed to a preservation agent. Some of the instant claims recite the invention more narrowly, as a mammalian cell instead of a cell, or in which the preservation agent is a non-metabolizable carbohydrate or sugar. But, even the claims that recite a method of preserving a mammalian cell also recite that the genus of any transporter protein may be used in the method. Thus, the claims recite two vast genera for each of which only a very few species are disclosed. The genus of a transporter molecule includes any type of molecule as well as all proteins- e.g.,

lipids, polysaccharides, polynucleotides, organic molecules. In the specification, however, the only transporter molecules or transporter proteins disclosed are the trans-membrane GLUT transporter proteins for monosaccharides and disaccharides. The genus of a preservation agent reads on any preservative, e.g., an antibiotic, insecticide, antioxidant, nitrite, sulphite, EDTA or formaldehyde. The only preservative agents disclosed in the specification are glucose and several non-metabolizable glucose analogues, sucrose, mannose, galactose and a hexose. A sufficient written description of a genus of cells, proteins or preservation agents (monosaccharides or disaccharides) may be achieved by a recitation of structural features common to each member (species) of the genus, **which features constitute a substantial portion of each member of the genus**. The structural features needed for the transporter molecule or protein to function in transporting the monosaccharide or disaccharide across a cell membrane are not recited. As noted above, the specification makes it clear that, for a preservation agent to function in the claimed method it must be structurally a mono- or disaccharide. Therefore, one skilled in the art cannot reasonably conclude that Applicants had possession of the claimed invention at the time the instant application was filed. This rejection was discussed in the previous Office action and in the interview with Applicants' attorney on April 29, 2008.

In their traversal, Applicants have cited case law but have not linked it specifically to the instant rejection. Applicants have not explained how this case law provides written description for the instant claims. In these cases, written description was considered to be sufficient for a method of adheredly applying one layer to another (because it was considered that one of skill in the art would know how to make one layer adhere to another); a method of using a mixture of a physiologically active steroid and DMSO (because a sufficient number of such steroids was known at the time of that invention); and a composition of inert fluid media (because it was

accepted at the time of that invention that gases were a type of fluid). But, the instant rejection is that the only transporter molecules or transporter proteins disclosed are GLUT transporter proteins and that the only preservation agents disclosed are several monosaccharides and several disaccharides. In the instant case, several species are not sufficient to support each of the claimed genera. Applicants note that their specification recites a "sucrose transporter protein" and a "mannose transporter protein," but two additional species of the genus of transporter molecules do not describe the remainder of the genus.

The instant claims were carefully considered in their entirety and words were not taken out of context. But, the instant claims are very broad, and the instant rejection is warranted in such a case, to indicate to Applicants what their claims read on. Applicants appear to argue that the claims are more limited than their words clearly indicate. But, the claims are examined as written, and, thus, no words have been taken out of context.

Applicants note that hepatocytes are the most difficult type of cells to preserve, but as discussed above, the claims are very broad and are not drawn to a method of preserving hepatocytes. Claims 10 and 21 do recite hepatocytes as one species in a widely varying Markush group of cell types that may be frozen, cells ranging from embryos to plant cells (which include seeds). Applicants' achievement, however, does provide written description for the scope of the claims.

Regarding the term preservation agent, again, the term was not taken out of context. The rejection is that many kinds of preservation agents are known, but the specification does not describe which transport molecules and which cells go with these preservation agents as a set. Because the corresponding transport molecules and cells are not described, the scope of the term must match the disclosure in the specification.

In view of the foregoing, the rejection of record is maintained with respect to the genera

of transporter molecule/transport protein and preservation agent. The portion of the rejection in connection with the term "biomaterial" is withdrawn, as this word has been amended to cells and deleted from the claims.

Claims 1, 8-15, 19-23, 26-28, 32-35, 37-39 and 43-45 are again rejected, and claims 58-60 and 62-64 are rejected, under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for the genera of:
1) a cell or mammalian cell as the biomaterial having a membrane that is preserved by the claimed method, 2) a glucose transporter protein or GLUT protein as the transporter protein or transporter molecule, and 3) glucose, a non-metabolizable glucose analogue, a hexose, sucrose, mannose or galactose as the preservation agent, does not reasonably provide enablement for the genera in the claimed method of any transporter molecule/protein and any preservation agent. As a result, the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to practice the invention commensurate in scope with these claims. This rejection was discussed in the previous Office action. As noted above, the term "biomaterial" has been amended to "cell."

Applicants assert that the Wands factors have not been applied correctly because their claims are narrow, not broad. Applicants note that their examples present the most difficult case and that it would be more predictable, rather than less, to preserve potato cells using the described sucrose transporters. Applicants note that they provide substantial guidance for applying the claimed method to other cells having membranes with transporter molecules that uptake preservation agents.

In reply, Applicants have not addressed the specifics of the rejection. The rejection is that because very few transport molecules are disclosed (GLUT, sucrose and mannose

transport proteins only) and because very few preservation agents are disclosed, particularly preservation agents that can be used with the disclosed transport molecules (a few monosaccharides and a few disaccharides only), it cannot be predicted that other species of these genera exist. Because of the lack of guidance in the specification, it would be undue experimentation to create the information and guidance needed to practice the scope of the claims. Applicants have not identified the undisclosed species of the claimed genera or indicated where the missing information and guidance can be found. Applicants have not addressed the portions of the rejection in connection with the genera of transport molecule/ transport protein and preservation agent that are not enabled by the specification. As discussed previously and above, Applicants' claims are very broad, because of these genera.

As for the most difficult case of preserving potato cells with sucrose, these studies appear to have been done in the prior art. They do not appear to be Applicants' experiments. Further, Toner et al. (US 6,127,177) note that mammalian cell membranes are not practically permeable to sucrose (see col. 4, lines 57-64).

As for providing substantial guidance for applying the claimed method to other cells having membranes with transporter molecules that uptake preservation agents, this comment appears to refer to methods of preserving cell types other than mammalian cells. This comment also does not address the portions of the rejection in connection with the genera of transport molecule/ transport protein and preservation agent that are not enabled by the specification. Nevertheless, similarly to the rejection above, the portion of the rejection in connection with the term "biomaterial" is withdrawn, as this word has been deleted from the claims.

In view of the foregoing, the rejection of record is maintained with respect to the genera of transporter molecule/transport protein and preservation agent, but is withdrawn with respect to the genus of the canceled term biomaterial. Replacing the word biomaterial with cell

overcomes this portion of the rejection.

Claim Rejections - 35 USC § 112, second paragraph

In view of Applicants' amendment to claim 15, this rejection is withdrawn.

Claim Rejections - 35 USC § 102

Claims 1 and 8-14 are again rejected under 35 U.S.C. 102(b) as being anticipated by Toner et al. (US 6,127,177), as evidenced by Gould et al. ("The glucose transporter family: structure, function and tissue-specific expression," Biochem J 295:329-341, 1993). This rejection was discussed in the previous Office action.

Applicants assert that Toner et al. do not teach the claimed method, because they do not use glucose as a preservation agent and because they use the technique of poration with *Staphylococcus aureus* alpha-toxin to transport sugars that are not naturally permeable to the cell membrane into the cells.

In reply, although Toner et al. use the method of poration with a bacterial toxin, the broad comprising language of the claims does not exclude treatment with a bacterial toxin to render the cell membranes porous. But, the steps in the claimed method may be amended to exclude this technique. Toner et al. disclose treating their cell suspensions with a variety of sugar solutions for cryopreservation, and one of the exemplary sugars is glucose (see col. 4, lines 25-28). As previously discussed, because cell membranes naturally have at least a dozen GLUT (glucose transport) proteins (because glucose is a major food source for the cells), when cells are contacted with a glucose solution, the glucose is taken up naturally. This feature is an inherent property of cells. Toner et al. disclose that trehalose, as a preservation agent in freezing, has the advantage that it is non-toxic and does not have to be removed from cells after

the cells are thawed, but the disadvantage that poration of the cell membrane with a toxin is required, because trehalose is not naturally transported into cells (see col. 2, line 65, to col. 4, line 42). Nevertheless, when cells are treated or contacted with glucose, the glucose is transported across the cell membranes.

In view of the foregoing, the record of rejection is maintained.

Claim Rejections - 35 USC § 103

Claims 1, 8-17, 19-23, 26-30, 32-35, 37-41, 43-45 are again rejected, and claims 58-64 are rejected, under 35 U.S.C. 103(a) as being unpatentable over Toner et al. (US 6,127,177) in view of Gould et al. ("The glucose transporter family: structure, function and tissue-specific expression," Biochem J 295:329-341, 1993); and Pescero et al. ("Glucose metabolism by trout (*Salmo trutta*) red blood cells," J Comp Physiol B 162:448-454, 1992. This rejection was discussed in the previous Office action.

Applicants have traversed the rejection, asserting that the claimed method requires that the preservation agent be loaded to a concentration sufficient for preservation using transporter molecules. Applicants have cited the first two sentences of the Summary of Pesquero et al. and assert that these sentences mean that there is no transporter molecule for transporting glucose analogues across trout erythrocyte membranes, because the uptake is so low. Applicants assert that the two references may not be combined because "it would frustrate the intent and purpose of Toner" and because there is no likelihood of success. Applicants assert that the prior art does not teach that cells can be successfully loaded to sufficient levels with non-metabolizable sugars. Applicants cite their experiments in which they showed that glucose and DMSO had harmful effects when used as cryoprotectants.

In reply, Pesquero et al. disclose that the purpose of their experiments was to determine

whether or not erythrocytes in fish (they studied trout) were similar to those in mammals with respect to glucose uptake and secretion. The first two sentences of the Summary mean that, as stated, there is no counterpart in trout to the mammalian transport system for glucose (the GLUT proteins) that was found in mammalian red blood cells. Pesquero et al. note that fish erythrocyte membranes are permeable to glucose, but that the uptake and equilibrium time vary among species (see p. 453, third full paragraph). Glucose penetrates the erythrocytes by a diffusion process (see p. 453, last paragraph).

Applicants, however, have read the references too narrowly, and, as previously discussed, Toner et al. were cited for their teaching of glucose as a cryopreservation agent (poration of the cells with a toxin not being necessary for cells that take up glucose naturally, as evidenced by Gould et al.). Pesquero et al. were cited for their teaching that non-metabolizable glucose analogues, such as OMG (3-O-methyl-glucose), when taken up by cells, are not metabolized to lactic acid, as glucose is. As previously discussed, glucose, as a food source, is the most widely used sugar in vertebrate cells, and vertebrates have a higher plasma concentration of glucose than of other monosaccharides. Glucose is metabolized in the glycolytic cycle to generate carbon dioxide, ATP and lactic acid (see p. 448). More acid, as lactic acid, is produced from glucose than from other glycolytic intermediates, even under aerobic conditions (see p. 450, left col.). Because OMG is not metabolized, it is detectable in experiments even after 1000 min. (see p. 449, Fig. 1). Because OMG does not produce acid, which one of ordinary skill in the art at the time of the invention would have known is not good for the health of freshly thawed cells, it would have been obvious to one of ordinary skill in the art at the time of the invention to replace the glucose with a non-metabolizable analogue, such as OMG, when using a glucose as a cryoprotective agent. This teaching of Pesquero et al. (that OMG does not produce acid in cells) provides the motivation to combine the two references. As

for an expectation of success, one of ordinary skill in the art at the time of the invention would have had every expectation of success in treating a sample of cells to be frozen with a sugar solution at a concentration of about 0.2-1.0 M in which the sugar is glucose, as disclosed by Toner et al. (see col. 4, lines 55-65; and col. 7, lines 25-42). The instant claims do not recite a minimum concentration for the preservation agent inside the cells at the time that they are frozen. The instant claims are not limited to any particular cells, although some claims recite mammalian cells. But, if Applicants' point is that fish cells do not take up as much glucose as mammalian cells, because the former lack transport proteins, the instant claims do not recite a method of preserving fish cells or non-mammalian cells.

As for the claimed method reciting that transport molecules are used to load a sufficient concentration of a preservation agent, as discussed above, Gould et al. disclose that the necessary transport molecules (GLUT proteins) for the preservation agent are present in mammalian cells, the claims do not recite a minimum amount of preservation agent or glucose that must be present in the cells before they are frozen, and Toner et al. disclose that glucose has a preservative and stabilizing effect on cells (biological material, see col. 4, lines 25-29).

As for the prior art not teaching that cells can be successfully loaded to sufficient levels with non-metabolizable sugars, again, the claims do not recite what a sufficient level is. Gould et al. disclose that for GLUT 2, which is a high-capacity, high-K_m transport protein in the liver, kidneys, pancreas and intestines, the K_m's for OMG and glucose are about 42 and 66 nM, respectively, values that are extremely close considering that they were measured in separate experiments by different researchers (see p. 330, first full paragraph). Thus, one of ordinary skill in the art at the time of the invention would have expected similar membrane transport properties for OMG and glucose. Also, as glucose is a known preservation agent for freezing cells, because non-metabolizable sugars (such as OMG) cross cell membranes, one of ordinary

skill in the art at the time of the invention would have expected to have been able to use a non-metabolizable sugar as a preservation agent.

As for the harmful effects of glucose and DMSO, as discussed above, the acid production from glucose is disclosed by Pesquero et al. The toxicity and cell lysis from DMSO are disclosed by Toner et al. (see col. 5, lines 15-17). Thus, these findings are not novel to Applicants.

In view of the foregoing, the rejection of record is maintained.

No claim is allowed.

Applicants' amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicants are reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Rosanne Kosson whose telephone number is (571)272-2923. The examiner can normally be reached on Monday-Friday, 8:30-6:00, alternate Mondays off.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's

supervisor, Nashaat Nashed can be reached on 571-272-0934. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Rosanne Kosson
Examiner, Art Unit 1652

rk/2007-05-12

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